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Response under 37 CFR § 1.116
--EXPEDITED PROCEDURE--
Examining Group 1600

THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Russell, et al.
U.S. Serial No. 09/043,665
Filed (U.S.): October 5, 1998

Entitled: Materials and Methods Relating to the
Transfer of Nucleic Acid Into
Quiescent Cells

Group:1632

Examiner: R. Shukla Ph.D

Attorney Docket No.: 4219/1340 (formerly 3789/81421)

Commissioner for Patents and Trademarks
Washington, D.C.20231

DECLARATION OF COLIN M. CASIMIR UNDER 37 C.F.R. 1.132

I, Colin M. Casimir, hereby declare that:

1. I am a co-inventor on the above-noted U.S. patent application; I received a Ph.D. from The University of Glasgow in 1981. I am currently a Senior Lecturer in the Department of Haematology at the Imperial College School of Medicine, in London, U.K. I perform basic research in the area of gene therapy, particularly for immunodeficiency. My research publications relating to gene therapy include the following: Thrasher, A., Chetty, M., Casimir, C.M., Segal, A.W. Restoration of superoxide generation to a chronic granulomatous disease derived B cell line by retrovirus mediated gene transfer. *Blood* 80,1125-1129 (1992); Thrasher, A., Segal, A.W., Casimir, C. M. Chronic Granulomatous Disease: Towards Gene Therapy. *Immunodeficiency* 4, 327-333 (1993); Povey J, Weeratunge N, Marden C, Sehgal A, Thrasher A, Casimir C. Enhanced Retroviral Transduction of 5-FU-Resistant Human Bone Marrow (Stem) Cells Using a Genetically Modified Packaging Cell Line. *Blood*

92, 4080-9 (1998); and Casimir, C. M. Prospects for Gene Therapy of Inherited Immunodeficiency. In: *Blood Cell Biochemistry*. Vol.. 8, *Hemopoiesis and Gene Therapy* Fairbairn, L.J. and Testa, N., eds Plenum Publishing Corp. (1999). I have served as an expert witness to the UK Government Committee on *In Utero* Gene Therapy and regularly review manuscripts for the academic journal *Gene Therapy*.

2. I have read the Office Action mailed in the above-referenced patent application on September 19, 2000, and understand that questions have been raised by the Examiner as to whether one of skill in the art would expect success in the treatment of a patient's disease according to the claimed methods.

3 The specification teaches a novel method for the stable introduction of nucleic acid constructs to quiescent cells that, due to their quiescence, are resistant to stable transformation. The methods of the invention achieve the stable introduction of a nucleic acid construct into quiescent cells, such as stem cells, which have previously been difficult to stably transfect because of their quiescence. The methods involve the steps of exposing quiescent cells expressing a growth factor receptor on their surface with a retroviral packaging cell line that expresses the cognate growth factor on its surface. Contact between the growth factor and its receptor triggers cell division in the quiescent target cell, making the target cell susceptible to stable transduction with the retrovirus made by the packaging cell line and encoding the therapeutic polypeptide. Following the transformation of target quiescent cells according to this method, the transformed target cells can be administered to a patient in need of the therapeutic polypeptide expressed by those cells.

The effectiveness of the method of introducing nucleic acid constructs to quiescent cells in vitro is demonstrated in the Examples presented in the specification of the application. Quiescent cells of a growth factor dependent cell line and hematopoietic progenitor cells from human umbilical cord blood were independently transduced with retrovirally-encoded β -galactosidase marker polypeptide in Examples 1 and 2, respectively. Staining for β -galactosidase activity demonstrates that the transduced sequence is expressed at detectable levels in the cells incorporating the retroviral genome

sequence. Pluripotent hematopoietic stem cells from adult human bone marrow were successfully transduced with retrovirally-encoded p47-phox sequence in Example 3.

4. Quiescent cells transduced in the manner described in the specification and claimed in claim 1 are no different, with respect to the expression of transduced sequences, than cells transduced by methods in the prior art. Once the target population of quiescent cells is transformed according to the methods disclosed and demonstrated in the specification, they are no different, with regard to their ability to express a therapeutic polypeptide encoded by the transduced construct, than other cells that have been transduced with a similar nucleic acid construct. The level of a therapeutic polypeptide expressed by a transduced cell is determined in large part by the activity of the promoter and other regulatory sequences responsible for driving expression from the construct. There is no reason to believe that the activity of such regulatory sequences will be different in cells transduced with a given construct according to the methods of the invention than in cells transduced with the same construct by standard means.

With regard to whether a therapeutically useful level of a therapeutic polypeptide can be expected, the level necessary for a therapeutic effect depends upon the disease being treated, and one does not have to achieve 100% of normal levels in order to treat a disease. Often, levels well below 100% can restore complete function. For example, Roos et al. showed that less than 10% of the normal level of NADPH oxidase is necessary for a normal phenotype in a human Chronic Granulomatous Disease (CGD) carrier (Roos et al, 1986, Heterogeneity in Chronic Granulomatous Disease. In: Progress in Immunodeficiency Research and Therapy II, eds. Vossen & Griscelli, p. 139, Elsevier, Amsterdam, The Netherlands; Exhibit A). Therefore, even levels of a therapeutic polypeptide well below those found in normal individuals can be therapeutically effective. See also Hirschhorn, R. Adenosine deaminase deficiency. *Immunodefic. Rev.*:2 175-198 (1990; Exhibit B). Given the severity of the targeted diseases (for example, untreated ADA deficiency is fatal, and CGD can be fatal), any treatment that abates the symptoms of the target disease should be considered a success.

5. Cells transduced with sequences encoding therapeutic peptides have been successfully used to treat disease. It was known in the art before the September 28, 1995 priority date of the present application that disease could be treated by administering cells expressing a therapeutic polypeptide. For example, Sekhsaria et al. showed that the administration of mature neutrophils and monocytes obtained by in vitro differentiation of peripheral blood hematopoietic progenitor cells transfected with a p47-phox cDNA efficiently corrected NADPH oxidase deficiency in human patients with Chronic Granulomatous Disease (Sekhsaria et al., Peripheral blood progenitors as a target for genetic correction of p47phox-deficient chronic granulomatous disease. Proc. Natl. Acad. Sci. U.S.A. 90: 7446-750 (1993; Exhibit C). Other studies demonstrating the treatment of disease using cells expressing a therapeutic polypeptide include, for example: The ADA human gene therapy clinical protocol. *Hum. Gene Ther.*:1 327-362 (1990; Exhibit D); Bordignon, C., Mavilio, F., Ferrari, G., Servida, P., Ugazio, A. G., Notarangelo, L. D., Gilboa, E., Rossini, S., O'Reilly, R. J., Smith, C. A. et al. Transfer of the ADA gene into bone marrow cells and peripheral blood lymphocytes for the treatment of patients affected by ADA- deficient SCID. *Hum. Gene Ther.*:4 513-520 (1993; Exhibit E) and Hoogerbrugge, P.M., van Beusechem, V. W., Kaptein, L. C., Einerhand, M. P., and Valerio, D., Gene therapy for adenosine deaminase deficiency. *Br. Med. Bull.*:51 72-81 (January 1995; Exhibit F).

6. Because polypeptides encoded by constructs introduced according to the methods of the invention are expressed in those cells, and because cells expressing similar constructs are known to be effective in the treatment of disease one may reasonably predict success in the treatment of a disease by administering cells transformed according to the methods of the invention. There is no reason to believe that the expression of transduced therapeutic gene sequences will be any different in cells transduced by the methods disclosed in the specification than in cells transduced by standard methods. Once a quiescent cell, previously difficult to stably transfect or transduce, is transduced as demonstrated in Examples 1-3, the expression of the transduced gene is dependent upon the expression control sequences used, not the method used to introduce the gene to the cell.. Therefore, so long as expression control sequences known to be active in the target cell are used to drive the expression of the therapeutic gene, it is predictable that a cell

transformed with the methods disclosed in the application will express therapeutically useful amounts of the therapeutic polypeptide. The method of introducing a therapeutic gene sequence to a quiescent cell disclosed in the application is well-suited for the transformation of stem cells, which tend to be quiescent *in vivo*. Therefore, the long-term proliferative potential of the transformed cell population can actually be much greater than that of non-quiescent cells transformed according to prior art methods because stem cells by their nature have nearly unlimited capacity to proliferate. The long-term maintenance or even expansion of transduced cells *in vivo* greatly enhances the therapeutic usefulness of transduced stem cells relative to non-stem cells for the treatment of human disease.

The retention of long term *in vivo* proliferative capacity by cultured stem cells has been demonstrated in numerous accounts in the literature including both animal models and human studies. While in some strains of mice, exposure of stem cells to certain cytokine combinations has led to a reduction in engraftment capacity, there is abundant evidence for the preservation of repopulating activity following retroviral transduction in mice, large animals and in humans.

In mice, see e.g., Bodine, D.M., Karlsson, S., and Nienhuis, A. W., Combination of interleukins 3 and 6 preserves stem cell function in culture and enhances retrovirus-mediated gene transfer into hematopoietic stem cells. *Proc. Natl. Acad. Sci. U.S.A.*:86 8897-8901 (1989; Exhibit G), and Szilvassy, S.J. and Cory, S., Efficient retroviral gene transfer to purified long-term repopulating hematopoietic stem cells. *Blood*:84 74-83 (1994; Exhibit H).

In large animals, see e.g., Bienzle, D., Abrams, Ogg AC; Kruth, S.A.; Ackland, Snow J.; Carter, R.F.; Dick, J.E.; Jacobs, R.M.; Kamel, Reid S.; Dube, I.D, Gene transfer into hematopoietic stem cells: long-term maintenance of *in vitro* activated progenitors without marrow ablation. *Proc. Natl. Acad. Sci. U.S.A.*: 91 350-354 (1994; Exhibit I), Bodine, D.M., Karlsson, S., and Nienhuis, A. W. 1989, Bodine, D.M., Moritz, T., Donahue, R. E., Luskey, B. D., Kessler, S. W., Martin, D. I., Orkin, S. H., Nienhuis, A. W., and Williams, D. A., Long-term *in vivo* expression of a murine adenosine deaminase gene in rhesus monkey hematopoietic cells of multiple lineages after retroviral mediated gene transfer into CD34+ bone marrow cells. *Blood*:82 1975-1980 (1993; Exhibit J), van

Beusechem, V.W., Bart Baumeister, J. A., Bakx, T. A., Kaptein, L. C., Levinsky, R. J., and Valerio, D., Gene transfer into nonhuman primate CD34+CD11b- bone marrow progenitor cells capable of repopulating lymphoid and myeloid lineages. *Hum. Gene Ther.*:5 295-305 (1994; Exhibit K), and van Beusechem, V.W., Bart Baumeister, J. A., Hoogerbrugge, P. M., and Valerio, D., Influence of interleukin-3, interleukin-6, and stem cell factor on retroviral transduction of rhesus monkey CD34+ hematopoietic progenitor cells measured in vitro and in vivo. *Gene Ther.*:2 245-255 (1995; Exhibit L).

In humans, see e.g., Dunbar, C.E., Cottler Fox, M., O'Shaughnessy, J. A., Doren, S., Carter, C., Berenson, R., Brown, S., Moen, R. C., Greenblatt, J., Stewart, F. M., et al., Retrovirally marked CD34-enriched peripheral blood and bone marrow cells contribute to long-term engraftment after autologous transplantation. *Blood*:85 3048-3057 (1995; Exhibit M), Brenner, M.K., Cunningham, J. M., Sorrentino, B. P., and Heslop, H. E., Gene transfer into human hemopoietic progenitor cells. *Br. Med. Bull.*:51 167-191 (1995; Exhibit N), and Brenner, M.K., Rill, D. R., Holladay, M. S., Heslop, H. E., Moen, R. C., Buschle, M., Krance, R. A., Santana, V. M., Anderson, W. F., and Ihle, J. N., Gene marking to determine whether autologous marrow infusion restores long-term haemopoiesis in cancer patients. *Lancet*:342 1134-1137 (1993; Exhibit O).

According to my understanding and in view of the extensive literature demonstrating the retention of long-term proliferative capacity by genetically modified stem cells, it was predictable as of September 28, 1995 that stem cells transformed using the methods described in the specification would retain proliferative capacity sufficient to permit treatment of a disease with those cells.

7. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that wilful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.



March 19, 2001

Date

Colin M. Casimir, Ph.D.